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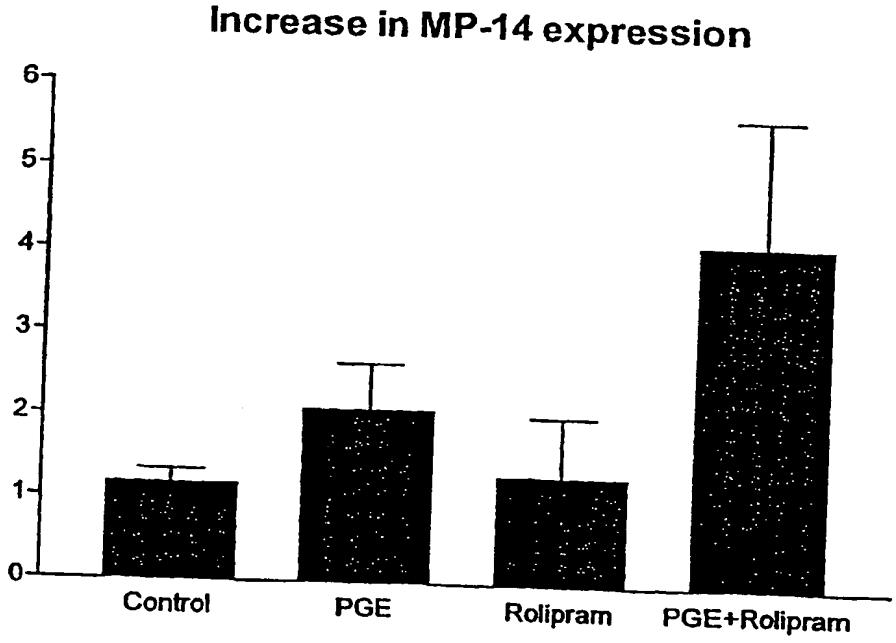
(54) Title: COMPOSITION COMPRISING PHOSPHODIESTERASE INHIBITORS FOR INDUCING CERVICAL RIPENING



(57) Abstract: A method of inducing cervical ripening in a patient, the method comprising administering to the patient a prostaglandin or agonist thereof and a phosphodiesterase (PDE) inhibitor.

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COMPOSITION COMPRISING PHOSPHODIESTERASE INHIBITORS FOR INDUCING CERVICAL RIPENING

The present invention relates to therapeutic systems and methods, and in particular it relates to therapeutic systems and methods for inducing cervical ripening.

The cervix acts as a barrier to the ingress of infection as well as a rigid barrier at the neck of the womb. This organ has relatively little muscle with the majority of the strength of the organ derived from collagen. In a normal, easy delivery, the cervix has to soften and stretch (eface) to allow the baby's head to pass through, if this can be achieved with little uterine contractility then subsequent contractions can deliver the baby with minimal trauma for mother and child.

Biologically active agents such as prostaglandins and chemokines are delivered to the cervix or vaginal fornix to aid cervical ripening for a number of reasons including (a) the induction of labour, (b) to soften an unfavourable cervix during labour, (c) to assist medical termination of pregnancy, (d) to assist uterine surgery and (e) to accelerate normal parturition and to reduce the accompanying risks and discomforts.

One of the drawbacks of existing methods of inducing cervical ripening is that excessive amounts of the cervical ripening agent, particularly prostaglandin, is administered which can lead to undesirable results. For example, prostaglandin gels used to ripen the cervix by administration to the vaginal fornix can stimulate the myometrium to contract excessively and, in extreme circumstances, an uterine rupture can occur (Maymon *et al* (1991) *Am J. Obstet. Gynecol.* **165**, 368-270).

Thus, it is desirable to find improved methods of inducing cervical ripening which make use of smaller amounts of cervical ripening agents, in particular smaller amounts of prostaglandin, so as to reduce the possible adverse effects found with previous methods.

The inventor now proposes that the use of a phosphodiesterase (PDE) inhibitor, in combination with a prostaglandin or agonist thereof, should achieve the desirable effect of reducing the amount of prostaglandin or agonist thereof required to induce cervical ripening to a useful extent. He has unexpectedly found that prostaglandin and a phosphodiesterase inhibitor have a synergistic effect on the expression of matrix metalloproteinase-14 (MMP-14) in human monocytes. MMP-14 can be considered to be a surrogate marker of cervical ripening as explained in more detail in the examples. Furthermore, the inventor has surprisingly found that prostaglandin leads to an increase in phosphodiesterase activity in monocytes which appears to be a direct negative feedback to reduce the effect of stimulation by prostaglandin.

The main receptors for prostaglandin E2 (PGE2) in the cervix (as shown in baboons) are the EP2 and EP4 sub-types; however, other receptor sub-types exist (namely EP1 and EP3). EP2 and EP4 receptors couple with adenylcyclase and use elevated cAMP as the messenger system. The levels of cAMP in tissue are governed both by synthesis and by catabolism by PDE. PDE can be blocked by specific inhibitors. The inventor believes, but without being bound by any theory, that the administration of a PDE inhibitor will enhance the effect of prostaglandin on EP2 (and EP4), which would avoid myometrial contractile effects which are mediated by EP1/EP3, which are not linked to adenyl cyclase but rather use other second messengers. Thus, the inventor believes that the effect is specific to PDE inhibitors in that the effect of prostaglandin (such as PGE) acting on its EP2

and EP4 receptors is to stimulate cAMP and the addition of the PDE inhibitor is a synergistic action. As noted above, the side effects of prostaglandin use are excessive myometrial contractions, but the receptors involved here are EP1. Any prostaglandin (such as PGE) reaching the myometrium will not synergise with the PDE inhibitor, since the EP1 and EP3 receptors do not use cAMP in signalling.

A first aspect of the invention provides a method of inducing cervical ripening in a patient, the method comprising administering to the patient a prostaglandin or agonist thereof and a phosphodiesterase (PDE) inhibitor.

The prostaglandin or agonist thereof and the PDE inhibitor may be administered in any order. Preferably, the PDE inhibitor is administered prior to administration of the prostaglandin or agonist thereof. It is particularly preferred if they are administered so that the PDE inhibitor can take effect in the cervix prior to administration of the prostaglandin or agonist thereof; however, the prostaglandin or agonist thereof and the PDE inhibitor may be administered substantially simultaneously, for example in the same composition.

It is preferred if the prostaglandin or agonist thereof is administered locally at the cervix, although as described in more detail below it may also be administered orally. The prostaglandin or agonist thereof may be administered as a gel or cream or in a vaginal pessary or vaginal tablet as is known in the art. It is particularly preferred if the prostaglandin or agonist thereof is administered to the cervix using a needleless injector. Details of administration of biologically active agents, including prostaglandin, to the cervix using a needleless injector described in our copending PCT Patent Application No GB02/00557, incorporated herein by reference.

The delivery may be directly to the cervix or may be *via* the vaginal fornix, which is a fold in the vagina where the cervix is located. Thus, the end of the needleless injector may occupy the vaginal fornix and delivery may be at or through the vaginal fornix into the cervix.

Typically, injections will aim at the cervix but may be aimed from different angles.

Preferably, the site of injection is "off-centre" into the cervical lumen or into any part of the externally presenting area of the cervix.

The needleless injector may be any suitable man-made needleless injector and, conveniently, may be a liquid injector or a powder injector. Thus, suitable needleless liquid injectors include those manufactured by Weston Medical Limited, Peterborough, UK and those described in WO 93/03779, WO 95/03844, WO 96/28202, WO 97/37705 and WO 00/10630, all of which are incorporated herein by reference.

Suitable needleless powder injectors include those manufactured by Powderject Research Limited, Oxford, UK and those described in WO 94/24263, WO 96/12513, WO 96/20022, WO 96/25190, WO 97/34652, WO 98/13470, WO 99/01168, WO 99/01169, WO 00/54827 and WO 00/62846, all of which are incorporated herein by reference.

It may be useful if the needleless injector has a "bent" configuration such that delivery of the prostaglandin or agonist thereof is to the cervix rather than to the walls of the vagina or to the entrance of the cervix. Thus, the needleless injector may be adapted for this purpose, and the geometry of the device arranged such that there is easy passage through the vagina but that the exit from the injector (ie outlet orifice) is angled such that the

prostaglandin or agonist thereof is delivered efficiently to the desired site on the cervix. The type of injectors described in WO 96/25190 may be particularly suitable; however, rather than the internal angle of approximately 90° shown in Figures 1 to 4 of WO 96/25190, it may be more appropriate if the internal angle of the head to the shaft is between 20° to 90°; preferably between 35° and 85°, for example 65°.

The arrangement of the head containing the outlet orifice shown in Figure 16B of WO 00/54827 may also be particularly suited for use in the present invention. Similarly, the arrangement shown in Figure 5 of WO 00/62846, where the exit plane is not perpendicular to the longitudinal axis of the nozzle, but angled, may be particularly suited for use with the invention.

The PDE inhibitor may be administered by any suitable route. The PDE inhibitor may reach the desired site of inhibition of PDE such as the cervix using many different routes of administration such as by application as a gel or using a suppository or pessary or vaginal tablet. Typically, in one embodiment, the PDE inhibitor is administered systemically. Suitable forms of systemic administration include oral. Many PDE inhibitors are orally available, so it may be convenient to administer the PDE inhibitor orally.

It is also convenient to administer the PDE inhibitor locally. Thus, the PDE inhibitor may be delivered locally at the cervix using, for example, a gel or cream or vaginal pessary or a needleless injector as described above in relation to the administration of the prostaglandin or agonist thereof. In preferred embodiments of the invention, the prostaglandin or agonist thereof and the PDE inhibitor may be combined in the same formulation for delivery simultaneously. Thus, the prostaglandin or agonist thereof and the PDE inhibitor may be combined in a gel or a cream or a vaginal pessary, or

combined in a needleless injector (or formulated together for use in a needleless injectors or formulated together in a vial for use in a needleless injector), and administered together to the patient.

The prostaglandin or agonist thereof may be any suitable prostaglandin or agonist thereof. By "prostaglandin or agonist" we mean any compound which acts as a prostaglandin agonist on a prostaglandin receptor. The prostaglandin agonist need not be a prostanoid. Typically, the agonist is one which binds the EP2 or EP4 receptor. It is preferred that the prostaglandin or agonist thereof is one which is able to induce cervical ripening when suitably administered to the female. It is preferred that the prostaglandin is a PGE or a PGI. Preferably, the prostaglandin is not a PGF or agonist thereof. It is preferred that the prostaglandin or agonist thereof is PGE_2 or a synthetic analogue thereof. Synthetic analogues include those modified at position 15 or position 16 by the addition of a methyl group, or those where the hydroxyl has been transposed from position 15 to position 16. Preferred examples of analogues of prostaglandin include Butaprost (an EP2 receptor agonist) and 11-deoxy PGE1 (an EP4 receptor agonist). For the avoidance of doubt, the term "prostaglandin" includes naturally-occurring prostaglandins as well as synthetic prostaglandin analogues.

Suitable prostaglandins or agonists thereof include, dinoprostone (sold as Propess by Ferring in Europe and Forest in the USA; sold as Prostin E2 by Pharmacia), gemeprost (sold by Farillon), misoprostol (which is sold as Cytotec by Searle and Pharmacia), alprostadil (which is sold as Caverject by Pharmacia and Viridal by Schwarz and MUSE by AstraZeneca) and limaprost.

Misoprostol is a PGE analogue which has EP2 and EP3 agonist effects. Its chemical structure if (±) methyl 11α, 16-dihydroxy-16-methyl-9-oxoprost-13-enoate.

An example of a non-prostanoid compound which acts as a prostaglandin agonist is AH23848, an EP4 receptor agonist.

EP2 agonists which may be useful in the practice of the invention include AH13205. Prostaglandin agonists are described in EP 1 097 922 and EP 1 114 816, incorporated herein by reference.

The 1-alcohol-19-hydroxy prostaglandin analogues described in US Patent No 4,127,612, incorporated herein by reference, may also be useful.

It is preferred that the prostaglandin is prostaglandin E_2 (PGE_2). Prostaglandins and agonists thereof, including PGE_2, are commercially available, for example from Pharmacia and Upjohn as Prostin E2.

The PDE inhibitor may be any suitable PDE inhibitor. Preferably, the PDE inhibitor is one which inhibits a PDE which is active in cAMP breakdown. The PDEs which are known to be active in cAMP breakdown are those of the types IV, VII and VIII. Preferably, the PDE inhibitors are selective for type IV or VII or VIII. By "selective" we mean that the inhibitor inhibits the particular type of PDE inhibitor for which it is selective more potently than another type. Typically, the selective inhibitor is around 5 to 50 times more potent an inhibitor of the selected PDE type than another PDE type. The selective inhibitor may be 100 or 200 or 300 or 500 or 1000 times more potent an inhibitor of the selected PDE type (eg type IV) than another PDE type. Typically, the selective inhibitor is 5 to 50 times (or even 100, 200, 300, 500 or 1000 times) more potent an inhibitor of the selected PDE type

than an inhibitor that is considered to be non-selective such as theophylline. Thus, theophylline is 30 times less effective than rolipram.

Non-specific PDE inhibitors include caffeine, theophylline, 3-isobutyl-1-methylxanthine (IBMX) and pentoxifylline (3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)-1H-purine-2,6-dione), although caffeine is not as active as the others and so is less preferred. The IC_{50} value for IBMX is 2-50 μM.

Specific (or selective) Type IV PDE inhibitors include rolipram (4-[3-cyclopentyloxy-4-methoxyphenyl]-2-pyrrolidinone) and Ro-20-1724 (4-[3-butoxy-4-methoxybenzyl]-2-imidazolidinone). The IC_{50} for rolipram is 800nM, and the IC_{50} for Ro-20-1724 is 2 μM.

CP 80 633 (Hanifin *et al* (1996) *J. Invest. Dermatol.* **107**, 51-56), CP 102 995 and CP 76 593 are also all potent type IV inhibitors (available from Central Research Division, Pfizer Inc, Groton, CT).

Another suitable PDE type IV selective inhibitor is denbufylline (1,3-di-n-butyl-7-(2-oxopropyl)-xanthine).

US Patent No 6,127,378, incorporated herein by reference, discloses phenanthridines substituted in the 6 position that are described as selective PDE inhibitors (mainly of type IV), that may be suitable for use in the methods of the invention.

It is particularly preferred if the PDE inhibitor is a PDE type IV selective inhibitor. Preferably, the type IV selective inhibitor is at least 2 times more potent an inhibitor of type IV PDE than another PDE type. More preferably, the type IV selective inhibitor is at least 5 times, 10 times, 20

times, 30 times, 40 times, 50 times, 100 times, 200 times, 500 times or 1000 times more potent an inhibitor of type IV PDE than another PDE type.

Preferably, selective inhibition is determined by a comparison of IC_{50} levels (Dousa (1999) *Kidney International* **55**, 29-62).

Other high affinity type IV selective PDE inhibitors include CPD 840, RP 73401, and RS 33793 (Dousa, 1999). The high affinity type IV selective PDE inhibitors have a K_i of approximately 1 nM while the lower affinity inhibitors have a K_i of about 1 μM.

The disclosures in Dousa (1999); Müller *et al* (1996, *Trends Pharmacol. Sci.* **17**: 294-298); Palfreyman & Souness (1996, *Prog Med Chem* **33**: 1-52); Stafford & Feldman (1996, *Annual Reports in Medicinal Chemistry* (vol 31) pp 71-80; Ed. Bristol, Academic Press, NY, USA); and Teixeira *et al* (1997, *Trends Pharmacol. Sci.* **18**: 164-171) relating to type IV PDE selective inhibitors are incorporated herein by reference.

Typically, using prior art methods, around 1mg of prostaglandin (typically PGE2) is administered in a vaginal gel to induce cervical ripening. It is believed that this amount of prostaglandin (such as PGE2) may be reduced to 500 μg or 200 μg or 100 μg or 50 μg or 20 μg or 10 μg in a gel when used in combination with a PDE inhibitor according to the invention. It is believed that the amount of prostaglandin (such as PGE2) may be reduced still further, perhaps to 25 μg or even 10 μg or lower, if it is administered using a needleless injector and in combination with a PDE inhibitor according to the invention. Thus, considerably lower doses of prostaglandin or agonist thereof may be used while still achieving a desirable level of induction of cervical ripening. It is believed that lowering the amount of

prostaglandin or agonist thereof administered will reduce the risks of undesirable side effects.

It will be appreciated that the combination of prostaglandin or agonist thereof and PDE inhibitor may be administered in different ways. One convenient way is to administer the prostaglandin or agonist thereof and the PDE inhibitor together locally in the same or different gels. Another convenient way is to administer the prostaglandin or agonist thereof as a gel to the cervix and to administer the PDE inhibitor orally. A still further convenient way is to administer the prostaglandin or agonist thereof to the cervix using a needleless injector and to administer the PDE inhibitor orally. A still further way of administering the combination is using a needleless injector for both the prostaglandin or agonist thereof and PDE inhibitor, either together or separately. Yet a still further way is to administer the PDE inhibitor as a suppository and the prostaglandin or agonist thereof as a vaginal gel or vaginal tablet.

In a further embodiment of the invention, the prostaglandin or agonist thereof is administered orally. In this embodiment it is also particularly preferred if the PDE inhibitor is administered orally.

In this embodiment it is particularly preferred that the prostaglandin or agonist thereof is a prostaglandin analogue which has been modified to reduce catabolism.

Such analogues include synthetic analogues that have been modified at position 15 or 16 by the addition of a methyl group.

Thus, one method of the invention makes use of the oral administration of a prostaglandin analogue which has been modified to reduce its catabolism

and which is orally available (such as misoprostol) and the oral administration of the PDE inhibitor, typically an inhibitor selective for Type IV PDE, such as rolipram. The advantages of oral administration is that it is much less invasive than intravaginal delivery.

The inventor believes that the combination of PDE inhibitor with the orally available prostaglandin or agonist thereof will mean that a lower dose of oral prostaglandin or agonist thereof will be required than in the absence of PDE inhibitor. It is believed by the inventor that this will have the advantage of reducing side effects caused by the oral prostaglandin, such as muscle cramps.

Typically, the dose of misoprostol given orally is 20 to 200 μg, for example 50 μg or 100 μg or 150 μg.

Misoprostol is both an EP2 and EP3 receptor agonist and consequently gives rise to unwanted uterine contractions. The inventor believes that misoprostol will be more efficacious as a specific cervical ripening agent in the presence of a PDE inhibitor.

As described above, it can be used orally in combination with a PDE inhibitor at a lower dose than in the absence of PDE inhibitor. It may also be used by directly applying to the cervix along with oral administration of PDE inhibitor. The effect of misoprostol on cervical ripening is believed to be weaker when administered orally compared to its administration directly to the cervix.

Typically, when a type IV PDE-selective inhibitor is administered orally, around 1 to 30 mg is used. Thus, a typical oral dose of rolipram or denbufylline is 1 mg or 5 mg or 10 mg or 30 mg. When a non-selective

PDE inhibitor is used, such as theophylline, and it is administered orally, the dose is between 5 and 50 mg, such as 5 or 10 or 20 or 30 or 40 or 50 mg.

When the PDE inhibitor is administered locally, for example in a gel, a dose of 10 μg to 2 mg of a selective type IV PDE, such as rolipram or denbufylline, may be used. Typically, the dose may be 50 μg, 100 μg, 500 μg, 1 mg or 2 mg. When a non-selective PDE inhibitor is used, such as theophylline, the dose administered locally, such as in a gel, may be 30 μg to 5 mg.

A second aspect of the invention provides the use of a prostaglandin or agonist thereof in the manufacture of a medicament for inducing cervical ripening in a patient wherein the patient is administered a PDE inhibitor. Thus, the patient may already have been administered the PDE inhibitor before administration of the prostaglandin or agonist thereof, or is administered the PDE inhibitor at the same time as the prostaglandin or agonist thereof or will be administered the PDE inhibitor after administration of the prostaglandin or agonist thereof.

A third aspect of the invention is the use of a PDE inhibitor in the manufacture of a medicament for inducing cervical ripening in a patient wherein the patient is administered a prostaglandin or agonist thereof. Thus, the patient may already have been administered the prostaglandin or agonist thereof before administration of the PDE inhibitor, or is administered the prostaglandin or agonist thereof at the same time as the PDE inhibitor or will be administered the prostaglandin or agonist thereof after administration of the PDE inhibitor.

A fourth aspect of the invention provides the use of a combination of a prostaglandin or agonist thereof and a PDE inhibitor in the manufacture of a medicament for inducing cervical ripening in a patient. Thus, the prostaglandin or agonist thereof and PDE inhibitor may be combined in the same medicament before administration to the patient.

The preferences for the prostaglandin or agonist thereof, PDE inhibitors, routes of administration, doses and so on for the second, third and fourth aspects of the invention are the same as for the first aspect of the invention.

A fifth aspect of the invention provides the use of a PDE inhibitor in the manufacture of a medicament for inducing cervical ripening. Before the present invention, no-one had suggested that a PDE inhibitor could be used in the context of inducing cervical ripening. Preference for the PDE inhibitor, route of administration, dose and so on is the same as for the first aspect of the invention.

A sixth aspect of the invention provides a therapeutic system for inducing cervical ripening, the system comprising a prostaglandin or agonist thereof and a PDE inhibitor. The therapeutic system may also be termed a "kit of parts".

Preferably, the therapeutic system contains a preferred prostaglandin or agonist thereof as defined in the first aspect of the invention. Still preferably, the therapeutic system contains a preferred PDE inhibitor as defined in the first aspect of the invention. The therapeutic system or kit of parts may suitably contain both the prostaglandin or agonist thereof and the PDE inhibitor packaged and presented in suitable formulations for use in combination, either for administration simultaneously or for administration which is separated in time. Thus, for example, in one embodiment where

the prostaglandin or agonist thereof and PDE inhibitor are for simultaneous administration locally to the cervix, the therapeutic system may contain a gel or cream or pessary or needleless injector which contains a combination of prostaglandin or agonist thereof and PDE inhibitor. Alternatively, in another embodiment where the prostaglandin or agonist thereof and PDE inhibitor are for separate administration in a particular treatment regime, the prostaglandin and PDE inhibitor are packaged or formulated separately. For example, the prostaglandin or agonist thereof may be formulated for administration using a needleless injector or in a vaginal pessary or cream or gel, and the PDE inhibitor is packaged or formulated for systemic administration such as oral administration.

The formulations of the prostaglandin or agonist thereof alone or PDE inhibitor alone or the combination thereof may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredients used in the invention with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration (eg of the PDE inhibitor or of a suitable prostaglandin or agonist thereof) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (eg sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

For local administration, for example to the vagina or cervix, it may be convenient to formulate the prostaglandin or agonist thereof and/or PDE inhibitor in combination with a dispersion agent or an agent which allows for increased transdermal or transmucosal or transepithelial transfer or penetration, such a dimethyl sulphoxide (DMSO) and the like. Suitable

agents are ones which are compatible with the prostaglandin or agonist thereof and/or PDE inhibitor (eg are solvents thereof).

Typically, a composition comprising a prostaglandin or agonist thereof and a PDE inhibitor is used in the practice of the invention. Preferably, the prostaglandin or agonist thereof is one which is preferred in the first aspect of the invention. Also preferably, the PDE inhibitor is one which is preferred in the first aspect of the invention.

The composition is typically packaged and presented for use in medicine. The composition may be used in human or veterinary medicine; preferably, it is used in human medicine.

Typically, the composition further comprises a pharmaceutically acceptable carrier. Thus, a pharmaceutical composition (or formulation as it may be termed) comprising a prostaglandin or agonist thereof, a PDE inhibitor and a pharmaceutically acceptable carrier is typically used in the practice of the invention. The carrier(s) must be "acceptable" in the sense of being compatible with the composition of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free. However, other pharmaceutical carriers which may be required for formulation for, and delivery by, a needleless injector may also be present. Similarly, the pharmaceutical composition may take the form of a gel, cream, suppository or pessary.

A seventh aspect of the invention provides a formulation of a PDE inhibitor adapted for delivery to the cervix. As noted, before the present invention, there was no reason to deliver a PDE inhibitor to the cervix and so no reason to formulate a PDE inhibitor so that it is adapted for delivery to the cervix. Thus, the invention includes formulations of a PDE inhibitor in a

gel or a cream or a pessary or a suppository for delivery to the cervix intravaginally. It is particularly preferred if the PDE inhibitor is formulated as a gel, for example a methyl cellulose gel, for example a methyl cellulose gel such as one containing between 0.5% and 2.0% methyl cellulose. Such a gel is also suitable for the administration of the prostaglandin or agonist thereof whether alone or in combination with the PDE inhibitor.

Similarly, before the present invention no-one had suggested the use of a needleless injector for the administration of a PDE inhibitor. Thus, an eighth aspect of the invention provides a needleless injector loaded for injection with a PDE inhibitor. The needleless injector may be a liquid injector or a powder injector, as is known in the art.

A ninth aspect of the invention provides a vial for insertion into, and containing an agent for delivery by, a needleless injector wherein the agent is a PDE inhibitor.

The term "vial" may be interchanged with "cartridge", "capsule" and the like, unless the context indicates otherwise. In particular, it includes the burstable membranes described in WO 94/24263 and the soft-walled capsules described in WO 96/12513.

Certain needleless injectors, for example those described in WO 93/03779, WO 95/03844, WO 00/10630, WO 94/24263, WO 96/20022 and WO 96/25190 are designed to receive pre-charged vials containing the agent to be "injected". Thus, the vials of this aspect of the invention include such vials pre-charged with an agent which is biologically active on the cervix, such as those described above and, in particular, cervical ripening agents. Accordingly, the vials are of the correct size and shape to fit into the appropriate needleless injector. Also, the vials are made of appropriate

material for their purpose. Suitably, the vial contains a unit dosage of the biologically active agent. Suitably, when used in a liquid needleless injector, the vial contains around 200 µl (for example, between 50 µl and 500 µl). Typical unit dosages are 10 to 200 µg for the prostaglandin or agonist thereof and 200 µg to 6.0 mg for the PDE inhibitor.

Suitable vials for use with certain needleless liquid injectors, and methods of manufacture and filling the same, are described in WO 00/15281, WO 97/36785, WO 97/22375, WO 96/19252, WO 96/15821, WO 98/12121 and WO 98/13086, all of which are incorporated herein by reference.

Suitable vials for use with certain needleless powder injectors, and methods of manufacturing and filling the same, are described in WO 00/54827, WO 00/62846, US 5,780,100, WO 98/21364, WO 99/01169, WO 96/20022, WO 96/12513 and WO 94/24263.

A tenth aspect of the invention provides a method of preparing a needleless injector for use in delivering a PDE inhibitor to the cervix, the method comprising loading the injector with the PDE inhibitor. Typically, the PDE inhibitor is loaded in a vial according to the twelfth aspect of the invention. The vial is then loaded into the injector. Alternatively, the injector is one which has a reservoir for the PDE inhibitor to be delivered, in which case the PDE inhibitor is loaded into the reservoir.

An eleventh aspect of the invention provides a pharmaceutical formulation comprising a PDE inhibitor and a carrier suitable for use in a needleless injector.

Typically, the pharmaceutical formulation is for use in a powder injector and the formulation contains particles of a density between about 0.1 and

about 25 g/cm^3 and of a size between 0.1 and 250 micrometres which particles comprise the said PDE inhibitor.

Further details of the use of needleless injectors and vials and formulations for use therewith may be found in, for example, in the Weston Medical and Powderject patent applications described above.

The methods, therapeutic systems, compositions and formulations and so on of the invention may be used to induce or aid cervical ripening for a number of reasons including (a) the induction of labour, (b) to soften an unfavourable cervix during labour, (c) to assist medical termination of pregnancy, (d) to assist uterine surgery and (e) to accelerate normal parturition and to reduce accompanying risks and discomforts. Thus, the methods, therapeutic systems, compositions and formulations and so on can be used for the induction of labour at term (ie time of ordinary birth), but it may also be used for the induction of pre-term labour, and induction of labour in connection with a pathological pregnancy, or in connection with intrauterine fetal death. It may be used to ripen dysfunctional cervices, ie when dilation stops before completion. The methods, therapeutic systems and compositions may also be used for preliminary cervical ripening prior to induction of abortion (eg in the first or second trimester abortion), and for induction of cervical ripening of a non-pregnant or pregnant female to assist surgical or diagnostic procedure such as D&C. Cervical ripening may also be induced in the female for the purposes of treatment by *in vitro* fertilisation.

The female on which the method or therapeutic system is used or to which the composition is administered is preferably a human female although the female may be any mammal such as a cat, dog, horse, cow, sheep, horse, pig and so on. The method may be especially useful for sheep where *in*

vitro fertilization and embryo transfer procedures currently involve laparoscopy which brings up ethical and animal husbandry considerations, the problem being that the cervix is impenetrable. It is believed that a diffuse injection of cervical ripening agent would give a very rapid response, allowing a wider use of *in vitro* fertilization.

The invention will now be described in more detail by reference to the following Figures and Examples wherein:

Figure 1 shows that prostaglandin and PDE inhibitor act synergisticaly to increase expression of matrix metalloproteinase 14 (MMP-14).

Figure 2 shows that prostaglandin increases expression of PDE type IV and that prostaglandin plus a PDE inhibitor further increases expression of PDE type IV.

Example 1: Prostaglandin and PDE inhibitor act synergistically to increase MMP-14 expression, a surrogate marker for cervical ripening

Matrix metalloproteinase-14 (MMP-14 or MT1-MMP) is a surface active MMP produced on a variety of cell surfaces, including monocytes. The importance of this enzyme is that it can proteolytically cleave pro-MMPs to active MMPs. MMP-14 is particularly good at cleaving pro-MMP-2 to MMP-2. MMP-2 is involved in converting procollagenase to collagenase and co MMP-14 can be considered to be a surrogate marker for cervical ripening which involves the production of collagenase. Furthermore, production of MMPs helps the infiltrating immune cells (eg monocytes) penetrate the cervix better.

Experimental methods

U 937 (human monocyte cell line) cells were grown in RPMI (PAA Laboratories) medium with 10% fetal calf serum added (PAA Laboratories). 2×10^6 cells per flask were treated with prostaglandin E_2 at 10^{-6} Molar or with Rolipram (4×10^{-6} Molar) for 20 hours.

After the incubation (20 hours), cells were pelleted and the mRNA was extracted with Tri-reagent (Sigma, Poole, UK). Total RNA was obtained by addition of chloroform and subsequent isopropanol precipitation. RNA was reverse transcribed with reverse transcriptase (Applied Biosystems) and random hexamers (Applied Biosystems). Probes and primers for MMP-14 were designed using Primer Express (Applied Biosystems) and were as follows:

Forward primer:

CCC CAG GCG ACT GCT CTA CT

Reverse primer:

TCG GGA CGA TGG GAA CAG

Taqman probe:

CCA GCG TTC CCT GCT GGA CAA GG

Template was amplified in a Taqman 7700 machine for 40 cycles using FAM/TAMRA dyes on the probe. The Applied Biosystems Kit was used to amplify and detect ribosomal (18S) RNA (using VIC/TAMRA dyes) as an internal control in the same reaction tube. After 40 cycles the Ct (related to cycle number at which signal appears) for the FAM and the 18S (VIC) were recorded and absolute relative quantitation was achieved using the formula $2^{-\Delta\Delta Ct}$ where Δ refers to the difference between the FAM and VIC signal related to an standard comparator included in each run.

Results

The results are shown in Figure 1. The combination of PGE (1 μM) and Rolipram (4 μM) is much better than either PGE or Rolipram alone in inducing MMP-14 expression. PGE and Rolipram appear to act synergistically.

Example 2: Prostaglandin increases expression of PDE type IV

The mRNA for phosphodiesterase IV-b was measured essentially as described in Example 1 above. mRNA was extracted after hours of incubation. The concentration of the PGE was 1 x 10^{-6} M and that of the 19-hydroxy PGE_2 was 5 x 10^{-6} M. The following primers and Taqman probe were used for quantitation of PDE IV b mRNA.

Forward
CCTTCAGTAGCACCGGAATCA
Reverse
CAAACAAACACACAGGCATGTAGTT
Probe
AGCCTGCAGCCGCTCCAGCC

Figure 2 shows that prostaglandin increases expression of PDE type IV in monocytes and that prostaglandin plus a PDE inhibitor further increases expression of PDE type IV. The increase in PDE activity follows both PGE and 19-hydroxy PGE application. This is a direct negative feedback to reduce the effect of the stimulus. PGE plus phosphodiesterase inhibitor increases PDE mRNA even further, but then synthesised PDE is nullified by the presence of the inhibitor.

This observation indicates that the presence of a PDE inhibitor should enhance the efficiency of PGE (by blocking PDE which breaks down cAMP), and it may explain why prostaglandin on its own is not every effective in cervical ripening in some women.

Example 3: Rectal Suppository

	mg/suppository
Rolipram	2
Hard Fat, BP (Witepsol H15 - Dynamit Nobel)	1770
	1772

One fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45°C maximum. The active ingredient is sifted through a 200 μm sieve and added to the molten base with mixing, using a silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45°C, the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250 μm stainless steel screen and, with continuous stirring, is allowed to cool to 40°C. At a temperature of 38°C to 40°C 2.02 g of the mixture is filled into suitable plastic moulds. The suppositories are allowed to cool to room temperature.

Rectal suppositories may be made which contain 5 mg or 10 mg of rolipram. The rectal suppositories are used in a patient who is also administered a prostaglandin.

Example 4: Pessaries

	mg/pessary
Rolipram or pentoxyfylline	0.1 to 20 mg
Anhydrate Dextrose	500
Potato Starch	493 (to 473)
Magnesium Stearate	7
	1000

The above ingredients are mixed directly and pessaries prepared by direct compression of the resulting mixture.

The pessary may also contain a prostaglandin such as Prostin E2 or misoprostol at a unit dose of between 10 and 200 µg.

Example 5: Gel formulation of PDE inhibitor

A methyl cellulose gel is prepared in water containing 1% methyl cellulose and Ro-20-1724 at a concentration of 2 mg/ml.

Example 6: Gel formulation of PDE inhibitor and prostaglandin

A methyl cellulose gel is prepared in water containing 0.5% methyl cellulose, rolipram at a concentration of 0.1 to 2 mg/ml and Prostin E2 at a concentration of 10 to 200 µg/ml.

Example 7: Gel formulation of PDE inhibitor and prostaglandin

A methyl cellulose gel is prepared in water containing 2% methyl cellulose, rolipram at a concentration of 0.1 to 2 mg/ml and misoprostol at a concentration of 10 to 200 µg/ml.

Example 8: Combined local and oral treatment

A female in need of cervical ripening is administered 10 mg rolipram orally followed after 1 hour by 200 µg of PGE_2 administered to the cervix in a 2% methyl cellulose gel.

CLAIMS

1. A method of inducing cervical ripening in a patient, the method comprising administering to the patient a prostaglandin or agonist thereof and a phosphodiesterase (PDE) inhibitor.

2. A method according to Claim 1 wherein the prostaglandin or agonist thereof is administered locally at the cervix.

3. A method according to Claim 1 or 2 wherein the PDE inhibitor is administered systemically.

4. A method according to Claim 3 wherein the PDE inhibitor and/or prostaglandin or agonist thereof are administered orally.

5. A method according to any one of the preceding claims wherein the prostaglandin or agonist thereof and the PDE inhibitor are administered simultaneously.

6. A method according to Claim 2 wherein the prostaglandin or agonist thereof is administered at or through the vaginal fornix into the cervix.

7. A method according to Claim 6 wherein the prostaglandin or agonist thereof is administered using a needleless injector.

8. A method according to any one of the preceding claims wherein the prostaglandin or agonist thereof is any one of a prostaglandin E, such as prostaglandin E_2 or an analogue thereof, dinoprostone, gemeprost,

misoprostol, alprostadil, limaprost, butaprost, 11-deoxy PGE1, AH23848, AH13205, 19-hydroxy PGE1 or 19-hydroxy PGE2.

9. A method according to any one of the preceding claims wherein the PDE inhibitor is selective for any of type IV, VII or VIII PDE.

10. A method according to any one of Claims 1 to 8 wherein the PDE inhibitor is any one of 3-isobutyl-1-methylxanthine (IBMX), pentoxifylline (3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)-1H-purine-2,6-dione), rolipram (4-[3-cyclopentyloxy-4-methoxyphenyl]-2-pyrrolidinone), CP80 633,, CP102 995, CP76 593, Ro-20-1724 (4-[3-butoxy-4-methoxybenzyl]-2-imidazolidinone), theophylline, caffeine, denbufylline (1,3-di-n-butyl-7-(2-oxopropyl)-xanthine), CPD 840, RP 73401 or RS 33793.

11. Use of a prostaglandin or agonist thereof in the manufacture of a medicament for inducing cervical ripening in a patient wherein the patient is administered a PDE inhibitor.

12. Use of a PDE inhibitor in the manufacture of a medicament for inducing cervical ripening in a patient wherein the patient is administered a prostaglandin or agonist thereof.

13. Use of a combination of a prostaglandin or agonist thereof and a PDE inhibitor in the manufacture of a medicament for inducing cervical ripening in a patient.

14. Use according to any one of Claims 11 to 14 wherein the PDE inhibitor is selective for any of type IV, VII or VIII PDE.

15. Use according to any one of Claims 11 to 14 wherein the PDE inhibitor is any one of 3-isobutyl-1-methylxanthine (IBMX), pentoxifylline (3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)-1H-purine-2,6-dione), rolipram (4-[3-cyclopentyloxy-4-methoxyphenyl]-2-pyrrolidinone), CP80 633, CP102 995, CP76 593, Ro-20-1724 (4-[3-butoxy-4-methoxybenzyl]-2-imidazolidinone), theophylline, caffeine, denbufylline (1,3-di-n-butyl-7-(2-oxopropyl)-xanthine), CPD 840, RP 73401 or RS 33793.

16. Use according to any one of Claims 11 to 15 wherein the prostaglandin or agonist thereof is any one of a prostaglandin E, such as prostaglandin E_2 or an analogue thereof, dinoprostone, gemeprost, misoprostol, alprostadil, limaprost, butaprost, 11-deoxy PGE1, AH23848, AH13205, 19-hydroxy PGE1 or 19-hydroxy PGE2.

17. Use according to any one of Claims 11 to 16 wherein prostaglandin or agonist thereof is administered locally at the cervix.

18. Use according to any one of Claims 11 to 17 wherein the PDE inhibitor is administered systemically.

19. Use according to Claim 18 wherein the PDE inhibitor and/or prostaglandin or agonist thereof are administered orally.

20. Use according to any one of Claims 11 to 19 wherein the prostaglandin or agonist thereof and the PDE inhibitor are administered simultaneously.

21. Use according to any one of Claims 11 to 20 wherein the prostaglandin or agonist thereof is administered at or through the vaginal fornix into the cervix.

22. Use according to any one of Claims 11 to 21 wherein the prostaglandin or agonist thereof is administered using a needleless injector.

23. Use of a PDE inhibitor in the manufacture of a medicament for inducing cervical ripening.

24. A therapeutic system for inducing cervical ripening, the system comprising a prostaglandin or agonist thereof and a PDE inhibitor.

25. A therapeutic system according to Claim 24 wherein the prostaglandin or agonist thereof is in a preparation for local administration at the cervix.

26. A therapeutic system according to Claim 24 or 25 wherein the PDE inhibitor is in a preparation for systemic administration.

27. A therapeutic system according to Claim 26 wherein the PDE inhibitor is in a preparation for oral delivery.

28. A therapeutic system according to any one of claims 24 to 27 wherein the system contains a needleless injector for administration of the prostaglandin or agonist thereof.

29. A therapeutic system according to any one of Clams 24 to 28 wherein the prostaglandin or agonist thereof is any one of a

prostaglandin E, such as prostaglandin E_2 or an analogue thereof, dinoprostone, gemeprost, misoprostol, alprostadil, limaprost, butaprost, 11-dexoy PGE1, AH23848, AH13205, 19-hydroxy PGE1 or 19-hydroxy PGE2.

30. A therapeutic system according to any one of Claims 24 to 29 wherein the PDE inhibitor is selective for any of type IV, VII or VIII PDE.

31. A therapeutic system according to any one of Claims 24 to 29 wherein the PDE inhibitor is any one of 3-isobutyl-1-methylxanthine (IBMX), pentoxifylline (3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)-1H-purine-2,6-dione), rolipram (4-[3-cyclopentyloxy-4-methoxyphenyl]-2-pyrrolidinone), CP80 633, CP102 995, CP76 593, Ro-20-1724 (4-[3-butoxy-4-methoxybenzyl]-2-imidazolidinone), theophylline, caffeine, denbufylline (1,3-di-n-butyl-7-(2-oxopropyl)-xanthine), CPD 840, RP 73401 or RS 33793.

32. A formulation of a PDE inhibitor adapted for delivery to the cervix.

33. A needleless injector loaded for injection with a PDE inhibitor.

34. A vial for insertion into, and containing an agent for delivery by, a needleless injector wherein the agent is a PDE inhibitor.

35. A method of preparing a needleless injector for use in delivering a PDE inhibitor to the cervix, the method comprising loading the injector with the PDE inhibitor.

36. A pharmaceutical formulation comprising a PDE inhibitor and a carrier suitable for use in a needleless injector.

37. Any novel method of inducing cervical ripening as herein disclosed.

38. Any novel therapeutic system for inducing cervical ripening as herein disclosed.

Figure 1

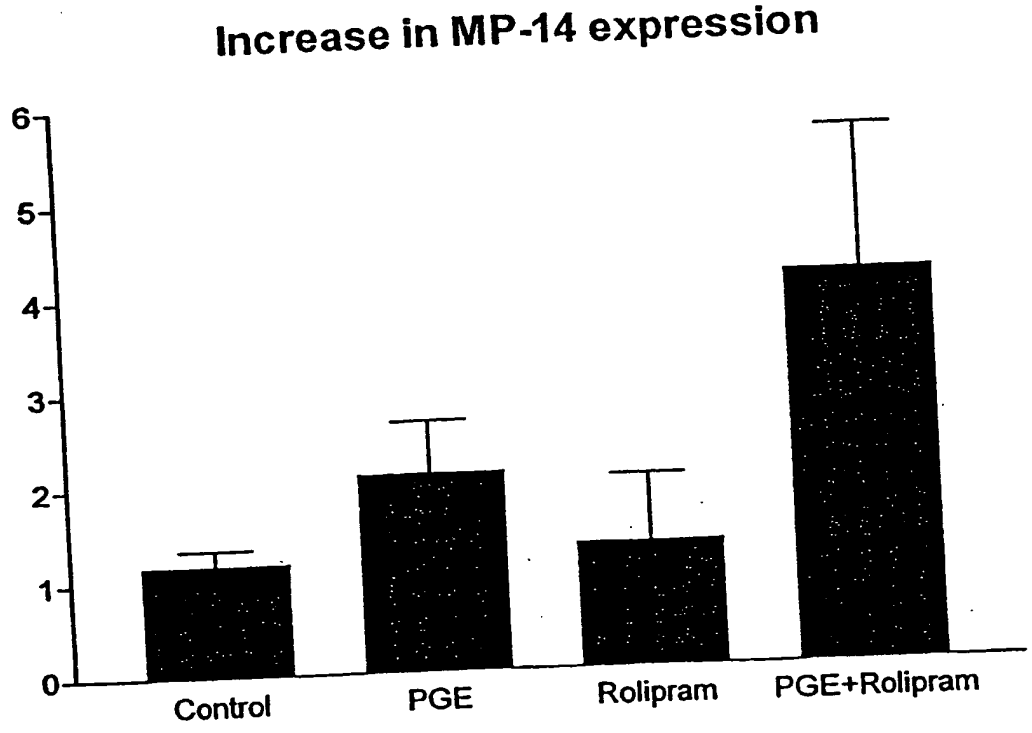
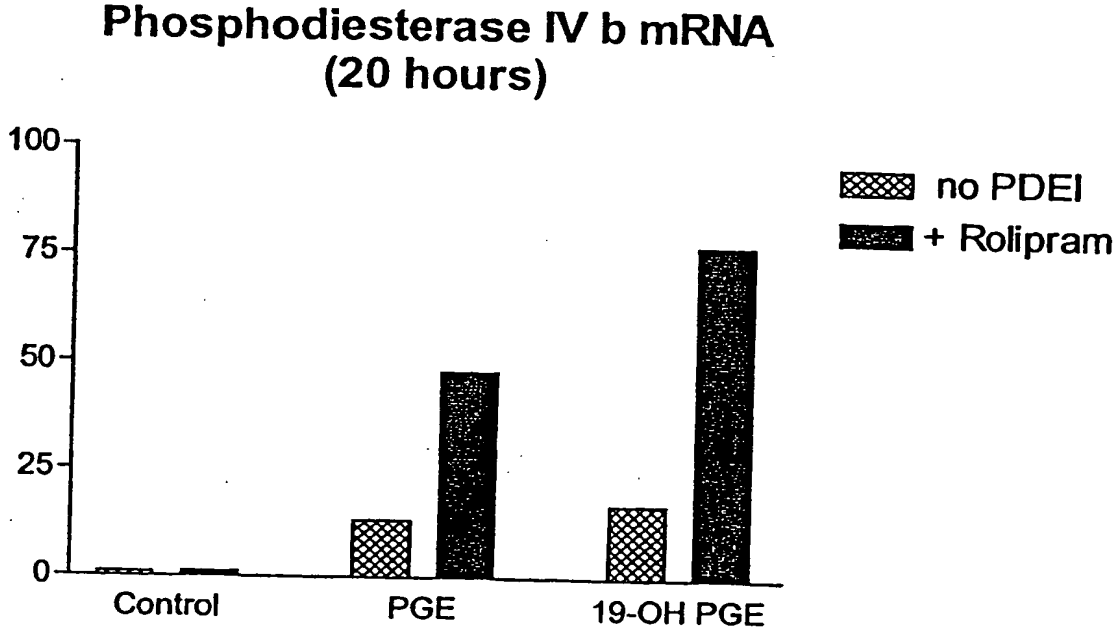

Increase in MP-14 expression
6
5
4
3
2
1
0
Control
PGE
Rolipram
PGE+Rolipram

Figure 2


Phosphodiesterase IV b mRNA
(20 hours)
100
75
50
25
0
Control
PGE
19-OH PGE
no PDEI
+ Rolipram

INTERNATIONAL SEARCH REPORT

PCT/GB 02/02085

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/5575 A61K31/52 A61K31/4015 A61K9/00 A61M5/30
A61P15/04 //(A61K31/5575,31:52),(A61K31/5575,31:4015)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, PASCAL, CANCERLIT, SCISEARC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 071 272 A (HOFFMAN ALAN S ET AL) 6 June 2000 (2000-06-06) claims 1,3,4; figure 1 column 3, line 20 - line 29 ---	32-38
Y		24-31
Y	KALIX P: "PROSTAGLANDIN E-1 RAISES THE CYCLIC AMP CONTENT OF PERIPHERAL NERVE TISSUE" NEUROSCIENCE LETTERS, vol. 12, no. 2-3, 1979, pages 361-364, XP001096387 EN ISSN: 0304-3940 page 362, paragraph 3 table 1 --- -/--	1-23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2002	13/09/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sindel, U

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NORSTROM A ET AL: "ADENOSINE 3' 5'-MONOPHOSPHATE IN RELATION TO INHIBITION OF CERVICAL SMOOTH MUSCLE ACTIVITY IN EARLY PREGNANT WOMEN" ACTA ENDOCRINOLOGICA, vol. 125, no. 2, 1991, pages 122-126, XP001096368 ISSN: 0001-5598 page 125, column 1, line 1-10 page 125, column 1, line 12-17 ---	1-31
Y	"Rote Liste 1992" , BUNDESVERBAND DER PHARMAZEUTISCHEN INDUSTRIE E.V. , AULENDORF XP002211186 Products 04 002 and 27 082 -----	26,27

INTERNATIONAL SEARCH REPORT

PCT/GB 02/02085

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		WO 9921607 A1	06-05-1999